

Volatile fatty acid findings in vaginal fluid compared with symptoms, signs, other laboratory results, and susceptibility to tinidazole of malodorous vaginal discharges

A M M JOKIPII,* L JOKIPII,† E VESTERINEN,‡ E PUROLA,‡ E VARTIAINEN,‡ AND J PAAVONEN§

From the *Department of Medical Microbiology, University of Turku, Turku, the Departments of †Serology and Bacteriology, and ‡Obstetrics and Gynaecology, University of Helsinki, Helsinki, and the §Department of Clinical Sciences, University of Tampere, Tampere, Finland

SUMMARY The relevance of volatile fatty acids as a diagnostic test in 79 women with abnormal vaginal discharge was evaluated by a blind, randomised, and placebo controlled trial of tinidazole as a single oral 2 g dose. Automated gas chromatography of ether extracts of discharges taken before treatment showed volatile fatty acids in 18. Volatile fatty acids correlated with malodour, colour, and microscopically assessed altered bacterial flora and clue cells. At follow up one week later, the odour, colour, and volatile fatty acids in the vaginal discharge of women treated with tinidazole had become normal more often than in those receiving placebo. The disappearance of volatile fatty acids correlated with clinically assessed improvement in women treated with tinidazole. The volatile fatty acid test as an indicator of anaerobic bacterial flora is objective, technically simple and fast, has few problems of sample size and transportation, and may be useful in the aetiological classification and follow up treatment of non-specific vaginal discharges.

Introduction

Malodorous vaginal discharge is a very common symptom and imposes a great demand on medical resources. It is aetiologically heterogeneous and in most cases none of the specific agents of vaginitis or cervicitis can be shown. Several bacterial causes have been suggested; including *Gardnerella vaginalis*,¹ anaerobic curved rods,² or increased numbers of mixed anaerobes.³ Attempts to define one specific disease have been made, but a simple microbiological definition has not been reached so far. Instead, the combination of a high pH, fishy odour, and clue cells in the vaginal discharge has been used to define a condition called bacterial vaginosis.

Anaerobic bacteria produce volatile fatty acids,⁴ and the detection of their metabolites in clinical material indicates anaerobic infection.⁵ Volatile fatty acids have been found in vaginal fluids of a large

proportion of women with bacterial vaginosis defined as above.³ The fact that metronidazole is effective in treating the condition^{6,7} further supports the implication of anaerobes. The present study was designed to mimic everyday gynaecological practice and to ignore any classification of patients beyond that based on the demonstration of one of the established specific microbial aetiologies — that is, to ignore so called anaerobic vaginosis based on presumptive tests. As an adjunct to a therapeutic trial of tinidazole,⁸ we evaluated the gas chromatographic detection of volatile fatty acids as a diagnostic test in a series of non-selected patients with malodorous vaginal discharge. The results were compared with symptoms, signs, other test results, and therapeutic outcome.

Patients, materials, and methods

PATIENTS

The study population consisted of 79 women who attended the department of obstetrics and gynaecology, University of Helsinki, with malodorous vaginal discharge. Patients were not selected any

Address for reprints: Dr Anssi M M Jokipii, Department of Medical Microbiology, University of Turku, Kiinamylynkatu 13, 20520 Turku, Finland

Accepted for publication 18 June 1985

further and, after giving informed consent, they entered a therapeutic trial of tinidazole. Detailed descriptions of the patients and the results of treatment in a subpopulation have been published separately.⁸

CLINICAL AND LABORATORY EVALUATION

Using a standardised interview we obtained data concerning menstrual history, contraception, sexual history, previous gynaecological diseases, and current symptoms. An unlubricated speculum was inserted into the vagina, and any evidence of vaginal or cervical infection and the characteristics of vaginal discharge were recorded. Specimens were taken for cervicovaginal Pap smear, cervical cultures for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, and vaginal cultures for *Trichomonas vaginalis* and *Candida* spp, using standard procedures as previously described.⁸ Sterile distilled water (2 ml) was then pipetted into the posterior vaginal fornix, and a cotton tipped swab was used to mix the remaining vaginal secretion with the water. The mixture was removed from the vagina with a pipette and injected into a test tube, which was tightly capped and stored at 4°C until gas chromatography was performed.

GAS CHROMATOGRAPHY

A measured volume of vaginal fluid was acidified using 18 N sulphuric acid and extracted with 1.5 ml of diethyl ether, which was transferred to a glass vial (HP 5080-8712, Hewlett Packard, Espoo, Finland), which was equipped with a few 1/16" pellets of crystalline sodium aluminosilicate (54005, Molecular sieve type 4A, BDH Chemicals, Poole, England) to remove water and closed with a cap of Teflon coated red rubber (HP 5080-8713) using a crimper (HP 8710-0979).

We used an automatic sample injector (HP 7672A) with 99 positions to apply 1 µl samples into a HP 5880A gas chromatograph equipped with a coiled glass column (1.83 m long with an inner diameter of 2 mm) packed with Chromosorb WAW 100/120 mesh with 10% FFAP and 1% hypophosphorous acid (Hewlett Packard). To control the sensitivity of detection, every tenth sample was an ether extract of a standard water solution of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, and caproic acids (Fluka, Buchs, Switzerland) at concentrations of 2×10^{-3} mol/l each. The injection port temperature was 200°C, the oven temperature was kept at 140°C for 2½ minutes and then raised to 170°C at a speed of 15°C a minute, and the flame ionisation detector was operated at 300°C. The carrier gas was nitrogen (at 30 ml/min) and the flame was generated by synthetic air (20% oxygen and 80% nitrogen at 400 ml/min) and hydrogen at 30 ml/min. The gas chromatography was operated with the aid of

a level four integrator terminal (Hewlett Packard). With the settings that were used, the threshold of detection of the fatty acids was about 10^{-4} mol/l in the original sample, depending on the sample size and the acid in question. The result of the sample was defined as positive when any of the acids from propionic to caproic was detected.

TREATMENT AND FOLLOW UP

Each woman was treated with 2 g of tinidazole (Tricanix; Neofarma, Helsinki) as a single oral dose or with identically packed placebo, and her sexual partner received the same treatment.⁸ The selection was randomised, a double blind design being used. None of the participants knew which treatment was being given, the clinical data were recorded without

TABLE I Relation between volatile fatty acids and various symptoms and other findings

	Volatile fatty acids		
	Present (n=18)	Absent (n=56)	p Value (X ²)
Symptoms			
Amount of fluid:			
Abundant	16	47	
Scanty	2	9	NS
Malodour:			
Strong	14	28	
Weak	4	28	< 0.05
Itching:			
Yes	6	29	
No	12	27	NS
Irritation:			
Yes	5	25	
No	13	31	NS
Signs			
Amount of fluid:			
Abundant	15	38	
Scanty	3	18	NS
Colour:			
Other than white	17	40	
White	1	16	< 0.05
pH:			
High	9	23	
Normal	9	33	NS
Cytological findings			
Bacterial flora:			
Coccoid or mixed	18	28	
Lactobacilli	0	28	< 0.001
Leukocytes:			
Abundant	15	35	
Scanty	3	21	NS
Clue cells:			
Present	15	23	
Absent	3	33	< 0.005
Microbiological findings:			
<i>Trichomonas vaginalis</i>	2	2	NS
<i>Candida</i> spp	1	6	NS
<i>Chlamydia trachomatis</i>	2	5	NS

knowledge of the laboratory results, the gas chromatography was performed using coded samples, and the results were not available to the other workers. The clinical and laboratory evaluations were repeated at the second visit one week later.

STATISTICAL ANALYSIS

To assess statistical significance, we used the χ^2 test or Fisher's test of exact probability, one tailed.⁹

Results

Complete laboratory investigations including gas chromatography for volatile fatty acids were

undertaken at the first visit of 74 women. In 18 cases (24%) volatile fatty acids were found in the discharge and their presence correlated appreciably with vaginal malodour, abnormal colour of discharge, altered bacterial flora, and clue cells (table I). Of 151 samples (from first and second visits) analysed by gas chromatography, 37 were found to be positive, and the same correlations were confirmed (data not shown). The occurrence of volatile fatty acids was independent of the presence of *Trichomonas vaginalis*, *Candida* spp, or *Chlamydia trachomatis* (table I). The patients with these specific cervicovaginal infections were excluded from subsequent evaluations of the effects of tinidazole.

TABLE II Effect of tinidazole treatment on variables of vaginal discharge

	Tinidazole group		Placebo group		Expected after tinidazole*	Tinidazole specific effect†	p value (χ^2)
	Before	After	Before	After			
Malodour:							
Strong	14	2	13	7	7.50		
Weak or none	11	23	10	16	17.50	4.93	< 0.05
Colour:							
Other than white	21	9	14	12	20.25		
White	2	14	9	11	2.75	11.45	< 0.001
pH:							
High	10	5	9	4	4.46		
Normal	12	17	11	16	17.54	0.87	NS
Bacterial flora:							
Coccoid or mixed	16	12	17	16	14.96		
Lactobacilli	10	14	9	10	11.04	1.58	NS
Leucocytes:							
Abundant	19	17	14	15	19.81		
Scanty	7	9	11	10	6.19	1.69	NS
Clue cells:							
Present	13	8	14	13	12.08		
Absent	11	16	12	13	11.92	2.02	NS
Volatile fatty acids:							
Positive	9	5	6	9	12.69		
Negative	19	23	21	18	15.31	3.81	< 0.01

* Expected assuming the change in ratio of positive: negative results in women receiving tinidazole would be proportionally the same as the observed change in those receiving placebo.

† Observed ratio of negative: positive results divided by the expected ratio after tinidazole treatment. The absence of tinidazole specific effect would give the result 1.00.

TABLE III Relation between clinical outcome and laboratory variables of vaginal discharge

	Clinical improvement* in women receiving:					
	Tinidazole		Placebo		Total	
	Yes	No	Yes	No	Yes	No
Volatile fatty acids:						
Remained detectable	0	5	0	4	0	9
Disappeared	4	1	1	1	5	2
p Value	0.024		NS		0.005	
Bacterial flora:						
Remained altered	6	7	6	15	12	22
Changed to lactobacilli	6	1	2	1	8	2
p Value	NS		NS		0.016	
Clue cells:						
Remained detectable	3	6	3	8	6	14
Disappeared	6	2	2	3	8	5
p Value†	NS		NS		NS	

* Judgement based on clinical signs before laboratory results were available.

† Fisher test of exact probability: $p > 0.05$ not significant.

In the women receiving placebo in the tinidazole trial the incidence of several symptoms and findings was reduced after treatment. The assessment of the effect of tinidazole was therefore based on calculations to adjust for the expected placebo effect (table II). Tinidazole specifically reduced the incidence of vaginal malodour, abnormal colour of discharge, and volatile fatty acids, whereas it had little or no more effect than placebo on pH, morphology of bacterial flora, and the presence of leucocytes or clue cells (table II).

The doctors classified each patient as improved or not improved before laboratory results were available. Sixteen of the 18 patients whose vaginal fluid contained detectable volatile fatty acids at the initial examination completed the follow up. Volatile fatty acids remained detectable in nine patients, none of whom improved clinically; the disappearance of volatile fatty acids correlated appreciably with clinical improvement, and this was more apparent in women receiving tinidazole than in those receiving placebo (table III). As to the other two laboratory variables that correlated with volatile fatty acids, normalisation of the coccoid or mixed bacterial flora to a predominance of lactobacilli correlated with clinical improvement, but the disappearance of clue cells did not (table III).

Discussion

Various tests for the diagnosis of non-specific vaginitis have been extensively evaluated. Our work differed from most in that consecutive patients with malodorous vaginal discharge were enrolled without selection. Self selection is necessarily subjective and may involve local bias; thus the objective data in table I should be helpful to compare our study population with that of other investigators. The design was necessary, as we wanted to evaluate the independent information provided by the gas chromatographic detection of volatile fatty acids.

Volatile fatty acids correlated with several abnormal characteristics of vaginal fluid. This agrees with the earlier finding that volatile fatty acids correlated with non-specific vaginitis as defined by the presence of two or more of the following characteristics of vaginal secretion: homogeneous quality, high pH, clue cells, and a fishy odour on the addition of potassium hydroxide.³ Thus the aetiologies leading to the various characteristics occur simultaneously or at least overlap to an appreciable extent. The microbial background of positive results in most tests is not known, but volatile fatty acids are probably metabolic products of anaerobes. Though we regard volatile fatty acids as evidence of the presence of anaerobes, we do not suggest that all the various test characteristics are necessarily influenced

by mixed anaerobes. Volatile fatty acid identification serves to classify vaginal discharges, and a proportion of false negative results is expected, as not all anaerobes produce distinctive volatile fatty acids.⁴

Test results do not necessarily indicate the aetiology of the disease. In the case of non-specific vaginal discharges, recent efforts have been focussed on the pragmatic approach, or finding associations between diagnostic characteristics and specific treatment. Only randomised, blind, and placebo controlled trials are adequate for this purpose, because most characteristics have subjective elements and because they may change without drug treatment, as evidenced by the present results. The volatile fatty acid test does not seem to have been evaluated in such trials before.

Although 90% cure rates have been reported,^{10, 11} we⁸ and others¹² regard a single oral dose of 2 g tinidazole to be less effective than a seven day course of metronidazole⁷ or a five day course of tinidazole.¹³ From the point of view of test evaluation, however, it was an advantage also to have treatment failures, though they were not expected when the trial was planned.⁸

Single dose tinidazole was found to eliminate within the one week follow up period the odour, abnormal colour, and volatile fatty acids, but not other variables of vaginal discharge, such as the microscopically assessed altered bacterial flora and clue cells. The tinidazole specific normalisation of volatile fatty acids correlated appreciably with clinical improvement. Thus in unselected patients the aetiological classification of vaginal discharges according to the volatile fatty acid test result seems to provide clinically important information.

The volatile fatty acid test is technically fast, as it takes some 15 minutes from the receipt of a specimen to the printed result, but its potential clinical use as a rapid diagnostic test is restricted to the immediate vicinity of a laboratory with the equipment and experience. Most of the time it will probably resemble various other tests that require the submission of a sample to the microbiology laboratory and awaiting the result to achieve an aetiological classification of vaginal discharges. The volatile fatty acid test is an approximation of demonstrating mixed anaerobes, by (semi)quantitative culture techniques, which are too laborious to become available for all patients with vaginal discharges and will remain as research tools. The demonstration of volatile fatty acids is more simple than the demonstration of non-volatile fatty acids^{3, 14} or amines,^{15, 16} two additional biochemical methods for identifying anaerobic flora. Compared with other simple presumptive tests, the volatile fatty acid test is objective and automatically documentable and has few requirements of sample transportation or size. To doctors who actually perform the bedside tests to define anaerobic vaginosis, the volatile fatty acid

test perhaps offers little more than objective documentation, but to those who do not test the pH, smell fluids treated with potassium hydroxide, or examine wet mounts for clue cells, the volatile fatty acid test seems to provide an alternative. The suggestion is realistic, because bacteriology laboratories are increasingly adopting gas chromatographic methods to identify anaerobes. It is quite obvious that the volatile fatty acid test did not identify exactly the same women as the previously reported test triad, but further studies are needed before it is possible to claim that one test or combination of tests is better than others in identifying the aetiology of malodorous vaginal discharge.

This work was supported by the Sigrid Jusélius Foundation, Helsinki, the Academy of Finland, and the Orion Pharmaceutical Company, Espoo, Finland. We thank Mrs Teija Johansson, Mrs Tarja Kakko, and Mrs Marja Teerimäki for technical help.

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